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Internal Fungous Parasites of Agriculture Seeds

IMPERIAL BUREAU OF MYCOLOGY

By Chunjen C. Chen

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Agricultural Experiment Station

Bulletin No. 240 November, 1920

INTERNAL FUNGOUS PARASITES OF AGRICULTURAL SEEDS

Chunjen C. Chen.

SUMMARY.

1. A review of the literature shows that a number of internal parasites have been reported in seeds of the following crops: Barley, beans, beet, bur clover, cabbage, clover, corn, cotton, cucumber, darnel, evening primrose, flax, grasses, hollyhock, pea, rice, sweet corn, timothy, tomato and wheat.

2. The morphological structures of the pathogens, in which they are reported to be carried within the seed, are: oospores of the Phycomycetes, uredo and teleutospores of the Uredinales, vegetative forms of bacteria, vegetative mycelium of fungi, "mycoplasma," and unknown forms.

3. It is recorded that pathogens introduced into seeds may enter the ovary with the pollen tubes, or grow into the seed through the tissues of the fruit.

4. Agricultural seeds collected from both diseased plants and healthy plants, seeds having diseased symptoms and seeds of normal appearance were used in the experiments herein reported.

5. A technique of sterilizing the seeds externally with an alcoholic solution of corrosive sublimate and of growing the sterile seedlings in test tubes with cotton saturated with nutrient solution and in agar plates has been developed for studying the internal seed-borne pathogens.

6. Washing the seeds in sterile water after treatment with alcohol has been found absolutely necessary in order to prevent the alcohol from injuring the seeds.

7. A species of *Cylindrophora* was isolated from sterilized asparagus seed.

8. *Alternaria sp.* was found in the seeds of cabbage.

9. Species of *Fusarium, Macrosporium* and *Alternaria* have been isolated from common beans which were sterilized externally. A species of bacteria, which gave a coral red colored pigment was also noted in the bean cultures.

10. A species of *Macrosporium* causing a rusty appearance on the roots of cowpea seedlings was observed.

11. Externally sterilized lima beans were found to be infected with a species of *Fusarium.*

12. Species of *Macrosporium* and *Fusarium* have been isolated from externally sterilized soybeans.

13. A fungous parasite of sweet corn seedlings was observed. It appears to be identical to *Oospora verticillioides* as described by Saccardo, which may be a conidial stage of a better known corn parasite.

14. The mosaic of tomato was not found to be transmitted through the seed.

15. *Rhizopus nigricans,* Ehr. was isolated from dark colored seeds of rotten tomato.

16. A sterile fungus was noted to be parasitic to wheat seedlings.

17. Seeds of barley, alfalfa, timothy, red clover and crimson clover were tested but no pathogens found.

18. Seed selection from disease-free plants, germination test, and hot water and hot air treatment of seeds are suggested as means for controlling internal parasites of seeds.

INTRODUCTION.

The problem of sowing healthy seed for the production of good crops has attracted considerable attention from various points of view for many years. However, the relation of internal seed infesting pathogens to the reduction of crop yields has not been generally recognized.

Some plant diseases most dreaded in farm crops are among those carried within the seed. Many of them have attained as wide distribution as the hosts themselves due to their concealment within the seed. There is no doubt that poor germination of seeds, missing plants in the field, shriveled grains, dying of young seedlings, poor yields, and dwarfing of plants are largely due to the action of pathogens which have infected the seed internally.

In the present Bulletin it has been the purpose to bring together and summarize the findings of previous investigators upon the subject, to develop a technique by which further studies can be easily carried out, and to add something from observations and experiments. Many difficulties have confronted the writer during the investigation, because our

knowledge of internal seed infesting pathogens is quite recent and also because no extensive or systematic investigation of seed diseases is known to have been previously made. Therefore, it is desirable to consider this Bulletin as a preliminary study on the subject.

Acknowledgment is made to Professor J. B. S. Norton, of the Department of Plant Pathology, at the University of Maryland, under whose direction the work has been done and whose kindly criticisms and suggestions have been most appreciated. Director H. J. Patterson, of the Maryland Experiment Station, who made this investigation possible by placing the writer as a research assistant of the Department of Plant Pathology, deserves many thanks.

HISTORICAL REVIEW.

Introductory.

The first credible report of an internal seed infesting pathogen is that of Frank (38, 39),[1] who in 1883 found the anthracnose (*Colletotrichum lindemuthianum*) in beans in Germany. In 1885 Smith (83) reported the presence of telia of *Puccinia graminis* in the pericarp of oat kernels. Six years later, 1891, Halstead (47) discovered the anthracnose in beans in America. In the following year, 1892, Beach (5) stated that the bean blight (*Pseudomonas phaseoli*) may be carried within the seed. These earlier workers have marked the opening of the record of internal seed borne diseases. More detailed historical accounts regarding previous findings on the subject are to be presented under separate host plants in the following section.

Pathogens Found Within the Seed of the Host Plant.

Barley (*Hordeum vulgare*).

The loose smut (*Ustilago nuda*) and rust (*Puccinia glumarum*) have been recorded as internal seed borne diseases of barley. They are to be discussed later under "Wheat."

Beans (*Phaseolus spp.*).

The anthracnose disease (*Colletotrichum lindemuthianum*) was demonstrated to be internally seed borne by Frank (38, 39, 52) of Germany in 1883. In America, 1891, Halsted (47, 49) found the spores of the anthracnose in the cavity between the cotyledons, and believed that the disease can exist through the winter, or from one growing season to another in the mature beans. This was confirmed by Beach (5) in 1892. Whetzel (90) in his studies with bean diseases in 1906 stated that the seed-coat of unripe beans is easily penetrated by the mycelium

[1]The serial numbers in parentheses refer to "Literature cited," at the end of this Bulletin.

and the fungus is directly established within. A diagram showing the relation of the anthracnose to the tissues of the bean was also given (90, p. 204).

The bean blight (*Pseudomonas phaseoli*) was first noticed by Beach (5) in 1892, and he believed that it is possibly carried within the seed. In the same year Halsted (48) reported that the germs are carried over from season to season in the beans themselves. In 1906 Whetzel (90) noted that the bacteria can gain entrance into the seed and remain there dormant over the winter.

Clinton (23) in 1905 presented evidence of the lima bean mildew (*Phytophthora phaseoli*) being carried within the seed in the form of mature oospores and mycelium.

In 1917 Harter (51) reported that the podblight of lima beans (*Diaporthe phaseolorum*) invades the seed and inhibits its germination. He also stated that the organism is easily isolated from the seed.

Reddick and Stewart (78) in 1919 published an interesting account of the transmission of the virus of bean mosaic in seed. They obtained over 50 per cent. of mosaic plants from seeds which were diseased and sterilized externally in chloride of lime for four hours.

Beet (*Beta vulgaris*).

That the beet rust (*Uromyces betae*) causing decrease of sugar content of the root may remain latent in the seed during the winter in the form of an intracellular "mycoplasm" was reported by Eriksson (35) in 1914.

Bur Clover (*Medicago sp.*).

The bur clover leaf-spot (*Cercospora medicaginis*) was reported by Wolf (91) in 1916 to be carried as viable conidia on the burs. However, recent studies made by Hopkins (59) in 1920 have given evidence of the fungous mycelium in the seed coats. He obtained pure cultures of the fungus from seeds which had been treated in mercuric chloride 1:1000 for thirty seconds.

Cabbage (*Brassica oleracea*).

The black-leg disease (*Phoma lingam*) of cabbage has been reported as being carried within the seed in the form of dormant mycelium by Henderson (54) in 1918. He was able to isolate the organism from the seeds after "(*a*) removed from the pod under aseptic conditions, (*b*) exposures of 3, 5, 6 and 10 minutes, respectively, to a 1:1000 bichloride of mercury solution, and (c) immersion in a 1:200 solution of 40 per cent. formaldehyde for twenty-one minutes." He also concluded that "the fungus penetrates the seeds, causing them to shrivel and darken perceptibly."

Clover (*Trifolium pratense*).

Bondarstev (15) in 1914 reported that the blossom mold disease of red clover (*Botrytis anthophila*) has been found within the seed coats in the form of mycelium. In 1916 Jaczewski (62) stated that the mycelium of the blossom mold fungus penetrates the clover seeds, thus transmitting the disease from year to year and making the control difficult.

Corn (*Zea mays*).

Burrill and Barrett (20) in 1909 considered that the fusarium rot of corn is carried inside the grain. They detected the white mycelium of the fungus all through the diseased grains. In 1916 Pammel, King and Seal (74) reported that the mycelium of *Fusarium* occurs abundantly in the embryo and endosperm of the corn grain.

Burrill and Barrett (20) in 1909 also reported that the mycelium of *Diplodia zeae* (Schw.) Lev., which causes ear rot of corn, penetrates the young tissue of the grain.

That *Fusarium moniliforme* (82) may be carried within the seed as the cause of a serious root infection of corn plants in the field was reported by Valleau (87) in 1920.

Cotton (*Gossypium hirsutum*).

That the anthracnose disease (*Colletotrichum gossypii*) is carried in the seed was reported by Barre (2, 3) in 1912. He stated that the fungus remains in the interior of the seed as dormant mycelium and is able to live as long as 12 to 15 months. In 1913 Gilbert (45) published an account of his experiment made in 1905, in which he demonstrated that the fungus remained in the seeds and later killed the seedlings.

Rolfs (79) in 1915 noted that cotton seeds may harbor the organism of the angular leaf spot (*Bacterium malvacearum*) in the inner tissues and when planted transmit the disease to the young seedlings. However, in a recent study made by Faulwetter (37) in 1919, he concluded that the internal infection of the seed occurs very rarely.

Cucumber (*Cucumis sativus*).

In 1915 Eriksson (36) in Sweden suggested that the diseases of cucumber caused by *Cladosporium cucumerinum*, *Cercospora melonis* and *Colletotrichum lagenarium* are internally carried through the seeds, involving the "mycoplasm" stage.

Darnel (*Lolium temulentum*).

Guerin (46) in 1899 noted the constant presence of a fungus in the form of mycelium in the interior of the seed. In 1902 Freeman (40),

after performing a series of experiments, ascertained the presence of the fungus in the embryo of the darnel seed; and in 1904 he (41, 42) again noted internal infection by this fungus. No name was given to the fungus, as its spores were not found.

Evening Primrose (*Oenothera sp.*).

Galippe (44) in his studies with the parasites of seeds reported in 1915 that "*B. anthracoides*" is contantly present in the seeds of the evening primrose.

Flax (*Linum usitatissimum*).

The flax canker (*Colletotrichum lini*) was considered as a distinctly internal seed disease by Bolley (11) in 1910. Pethybridge and Lafferty (75) in 1918 reported that the fungus (*Colletotrichum linicolum*) causing the yellowing or damping-off of flax seedlings hibernates as mycelium in the epidermis of the seed coat. In the same year it was noted (1) in Ireland that a *Colletotrichum* causing a disease of flax seedlings hibernates in the skin of the seed and infects the plant while very young. The same fungus was probably referred to by different writers.

Grasses (*Gramineae*).

The occurrence of rust spores in the interior of seeds of grasses (*Bromus mollis* and *Agropyron spp.*) was noted by Beauverie (6) in 1913. He (7, 8) again in 1914 reported that the fruiting bodies of the rust within the seed coats of grasses aid greatly the dissemination of the epidemic. See also "Wheat."

Hollyhock (*Althea rosea*).

Eriksson (32) in 1911 reported that the rust of hollyhock (*Puccinia malvacearum*) may be carried in the seed. He (33) also stated, according to his "mycoplasm" theory, that the fungus passes the winter in symbiotic relations with the seed. In 1912 and 1914 Blaringhem (9, 10) of France made the same report, and thought the relation of symbiosis had become an acquired character of the host.

Oat (*Avena sativa*).

In 1885 Smith (83) found telia of *Puccinia graminis* in the pericarp of oat grain and also thought that teliospores are carried inside the aleurone layer of the grain.

Pea (*Pisum sativum*).

That the blight of peas (*Ascochyta pisi*) is an internally carried disease was first considered by Halsted (50) in 1893. Later in 1895

Krueger (63) noted the fungous mycelium growing out from pea seeds which had been soaked in water for 48 to 72 hours. Van Hook (88) in 1906 stated that the fungus grows through the husk of infected pods into the seed and produces spores there.

Dorothy Cayley (22) of England in her studies with a new bacterial disease of *Pisum sativum* reported in 1913 that the disease organism is transmitted in the interior of the seed.

Rice (*Oryza sativa*).

In 1918 Nishikado and Miyake (68) of Japan recorded the possibility of the brown spot disease (*Helminthosporium oryzae*) being carried within the rice grains.

Sweet Corn (*Zea saccharata*).

That the grains of sweet corn contain the germs of the wilt disease (*Pseudomonas stewartii*) was first reported by Stewart (85) in 1897. Later, Cook (24) in 1918 stated that the bacterial wilt carried in the seed from year to year is the chief means of dissemination.

Timothy (*Phleum pratense*).

That the leaf smut (*Ustilago striæformis*) may pass the winter in the embryo of the seed was demonstrated by Osner (73) in 1916. He noted that the smut enters the embryo of the seed, in the form of mycelium, through blossom inoculations.

Tomato (*Lycopersicum esculentum*).

Westerdijk (89) in her report on experiments with the tomato mosaic in Holland in 1910 has claimed that the disease is hereditary through the seed.

Wheat (*Triticum æstivum*).

In 1895 and 1896 Maddox (64, 65) of Tasmania noted the first evidence of internal seed infection in the loose smuts of wheat and barley (*Ustilago tritici* and *Ustilago nuda*, respectively). He stated that the ovaries of the wheat in the flowering stage can be artificially inoculated, and the grains developed from these infected ovaries produce smutted plants. Seven years later, 1903, Brefeld (18) observed the same occurrence. Hecke (53) in 1904 and Brefeld with his co-worker Falck (19) in 1905 have given a detailed account showing that the smut mycelium enters the ovary of the host plant and remains dormant in the mature grain. In 1907 Hori (60) reported the same result in his experiments in Japan. In America, Barrus (4) in 1908 and Freemen and Johnson (43) in 1909 published their conclusions in favor of previous in-

vestigators. Recently in 1918 Miura (67) of Japan has noted that the smut spores on the stigma of wheat and barley penetrate the tissue, and the fungus also was found in the embryo in the form of mycelium two or three days after the germination of its spores.

That the rust of grains may be transmitted within the seed through the dormant mycelium was the belief of Smith (84) in 1886. However, Eriksson (29) in 1897 advanced the mycoplasm theory for the transmission of the rust of wheat and barley (*Puccinia glumarum*). Zukal (92) in 1899 supported the dormant mycelium theory of Smith, and concluded that the mycelium lived over in the seed and grew into the embryo through the scutellum and developed with the plant. In the same year, Massee (66) developed the seed-borne spore theory. Later in 1914 Beauverie (7) found the spores of *Puccinia glumarum* within the seed coat of barley.

A conclusion was drawn by Eriksson (30) in 1898 that the common rust of wheat (*Puccinia graminis*) is inherited from the mother plant in the seed grain and lives there in a latent form which he called "mycoplasma." He (31) again in 1902 claimed that cereal rusts cannot be propagated either by an intercellular mycelium, or by groups of spores on the surface or in the interior of the grain. He further claimed that the uredo and teleutospores are not abundant enough to cause a habitual infection. However, in 1905 and 1906 Bolley and Pritchard (13,14) found both uredospores and teleutospores of the rust beneath the bran layer of the grain, and believed that the disease might be transmitted in this manner. Pritchard (76, 77) in 1911 presented evidence that the rust may be transmitted as dormant mycelium in the seed. In 1914 Eriksson (34), referring to an article by Beauverie (6) and the report by Pritchard (77), stated that he had reported the occurrence of rust spores in seeds of cereals in 1896, but that he considered it abnormal and not of practical importance in the propagation of the rusts.

Hungerford (61) in 1920, after a careful and extensive experiment, reported that the stem rust is not transmitted from one wheat crop to the next through the infected seed grain; although the uredinia and telia have been found embedded in the pericarp of wheat kernels.

The wheat scab (*Fusarium roseum*) was proved by Selby and Manns (81) in 1909 to be carried within the wheat kernel as an internal parasite.

In a study of the fungus flora of the straw and seed of wheat in 1913, Bolley (12) made the following statement: "They are *Alternaria, Macrosporium, Helminthosporium, Acrothecium, Colletotrichum, Fusarium* and *Cephalothecium*. Each of these can be obtained from the seed coats and interior of the grains from this section and from wheat samples from almost any wheat growing region in the world."

In 1915 O'Gara (72) reported a new disease of germinating wheat (*Podosporiella verticillata*) destroying the contents of wheat kernels at or near the time of germination. He also found the fungus producing no

growth external to the wheat kernel, being wholly within the epidermal covering.

Forms in Which Pathogens May Be Carried in the Seed.

The morphological structures or forms of the pathogens, in which they are carried within the seed, are highly important for the student of internal parasites of seeds to know. Based upon the pervious findings the following categories [1] have been made:

A. Sexual resting spores.
   1. Oospores of the *Phycomycetes*—for example, *Phytophthora phaseoli* of lima beans (23).
   2. Uredo and teleutospores of the *Uredinales*—for example, *Puccinia graminis* (13, 14).

B. Asexual bodies.
   1. Vegetative forms of bacteria—for example, *Pseudomonas stewartii* of sweet corn wilt (85).
   2. Vegetative mycelium of fungi—"The mycelium of a number of pathogenic fungi may invade the ovaries of the host without any marked or even evident injurious effect upon maturing seed. The intercellular mycelium in this condition suspends active growth during the dormant or resting period of the seed to resume active growth with the germination of the seed through the sprout of which it invades the growing plant." For example, *Colletotrichum lindemuthianum* of beans (90), *Phoma lingam* of cabbage (54), *Botrytis authophila* of red clover (15), *Colletotrichum gossypii* of cotton (2,3), *Ustilago striaeformis* of timothy (73), *Ustilago tritici* (19, 60), *Ustilago nuda* (19, 60), and *Puccinia graminis* of wheat (76, 77).

C. "Mycoplasma"—According to Eriksson, a fungus may exist in the seed in a "mycoplasma" form which serves as the means of transmission from crop to crop. For example, *Puccinia graminis* of wheat (30), *Puccinia glumarum* of wheat and barley (30), and *Uromyces betæ* of beet (35).

D. Unknown form (physiological virus)—for example, bean mosaic (78) and tomato mosaic (89).

In regard to the question of how the parasites may be introduced into the seeds, Whetzel (90) has stated that unripe beans are easily penetrated by the anthracnose mycelium and the fungus is directly established within. Henderson (54) reported that the black-leg fungus is able to penetrate cabbage seeds, causing them to shrivel and darken. Van Hook (88) noted that *Ascochyta pisi* grows through the husk of infected pea pods into the seed. Galippe (44) in 1915 stated that parasites very likely gain entrance to the ovary with the pollen tubes. He

[1] In compiling these a section of "forms and conditions in which pathogens may be distributed," in the "reading outlines" of plant pathology, by H. H. Whetzel of Cornell University, has been freely consulted.

also reported that the wind not only carries the pollen grains but also other foreign particles collected on the way, alive or inanimate, and organic or mineral. He found the parasites in the anthers, on the pollen, in the interior of the style and ovary, and on the stigma. Therefore, he concluded that the parasites must have been introduced into the seed at the time of fecundation. The loose smut of wheat and barley is a good example (64, 65, 67).

PRESENT INVESTIGATIONS.

Materials.

The present investigations have been confined mainly to procuring some information regarding some fungi, whether saprophytic or parasitic, that are carried in the seed of agricultural plants. Seeds collected from both diseased plants and healthy plants, and seeds having diseased symptoms as well as seeds of normal appearance were used in the experiments. Pathogens isolated from these seeds, in which they may be harbored, have been carefully studied to determine their parasitism to the seedlings of host plants.

Development of Technique.

The technique for the study of internal seed diseases has never been standardized, for the methods are still in the experimental stage, and no extensive or systematic investigation of seed diseases has yet been made. However, a good general technique will be indispensably needed when such studies are made. In the present study, the development of the method is, therefore, emphasized with a view to paving the way for more practical results, as well as to serve as an easy means for attacking the problem of internal seed diseases. This method has been developed from the one used by Selby and Manns (81) in their studies on diseases of cereals and grasses. It seems desirable to give their own description of the technique employed by them, before taking up for consideration the writer's individual work. The details of their method follow:

"The grain is soaked in water 8 to 10 hours (preferably over night) to soften or germinate the spores most resistent to fungicides. The seed is then immersed for one to two minutes in alcohol (50 per cent.) containing two grams of corrosive sublimate in each 1000 cc. This solution is poured off and the seed covered with commercial alcohol, 93 per cent. (absolute alcohol is less liable to contain germinable spores) to remove traces of the fungicide; this latter operation is repeated. This treatment can best be carried out in flasks or tubes properly plugged. The surface sterile kernels can then be picked out with sterile forceps and placed on agar in Petri dishes; the latter should be placed in a moist chamber to aid the germination of the grain. Kernels which are found sterile upon germination may be removed by sterile forceps and placed in sterile soil or in sterile tubes for further study.

Cultures were made of wheat plants in tubes, of which the lower portion, one inch in length, was filled with absorbent cotton and moistened with tap

water. After being sterilized these served as an efficient means both of learning whether the kernels were infested with internal parasitic fungi and of the effect of these parasites in the seed upon the young plant."

The technique developed by the writer (70) and used in the experiment is outlined as follows:

1. Soak the seeds to be studied in water for 10 to 12 hours in room temperature. The presoaking is to render the spores of pathogens on the seed coats more susceptible to the action of the disinfectant, and at the same time to prevent seed injury by the disinfectant. The efficiency of this treatment has also been tested out by Braun (16, 17).

2. Shake the presoaked seeds from 2 to 5 minutes in an alcoholic solution of corrosive sublimate (2 grams to 1000 cc. of 50 per cent. alcohol) in a sterilized test tube.

3. Remove the traces of the disinfectant from the seeds by first washing once in ethyl alcohol (93 per cent). and then three times in sterile water.[1] The writer has determined by a series of experiments that the latter operation is absolutely necessary in order to prevent the alcohol from injuring the seeds. In the tests summarized in the following table, one hundred seeds of each kind presoaked in water for 10 hours were used for each trial. They were given the standard germination test in the State Seed Laboratory. The table shows the details and results.

Treatment.	Percentage of Germination.			
	Wheat.	*Corn.*	*Peas.*	*Soybeans.*
No treatment	72.5	88	92	92.5
Immersed in corrosive sublimate (alcoholic) solution for 5 minutes	0	74.5	58	89
Immersed in corrosive sublimate (alcoholic) solution for 5 min. and washed in 93% alcohol	0	20	11	53
Immersed in corrosive sublimate (alcoholic) solution for five minutes; then washed in 93% alcohol; then washed in water 3 times.	60.5	91	77	90.5

The percentages in the table represent the average of two duplicate tests for each kind of seed.

[1]There is possibility of contamination in making several washings, but no evidence was found by the writer in his work.

Probably the alcohol washing could be eliminated, but it is desirable to remove any chance of interference with the growth of internal organisms.

PLATE I.

Shows the method of obtaining sterile seedlings by planting seeds externally sterilized in Petri dishes containing sterile, nutrient agar. Natural size.

4. Pick out the surface sterilized seeds with sterile forceps and plant them in sterile nutrient agar plates (Plate I) for germination, during which many of the pathogens carried internally will develop sufficiently to be recognized and can then be isolated for further examination.

Or, the surface sterilized seeds may be planted in large thick test tubes containing absorbent cotton saturated with a sterile nutrient solution (Plate II). Thus we are able to learn the presence of internally carried parasites of the seed, and to actually determine their effect upon the young plant. The nutrient solution enables the seedlings to continue their growth, so that death from lack of nutrient may not be

confused with the action of parasites. The cultures may be placed in a moist chamber to aid germination.

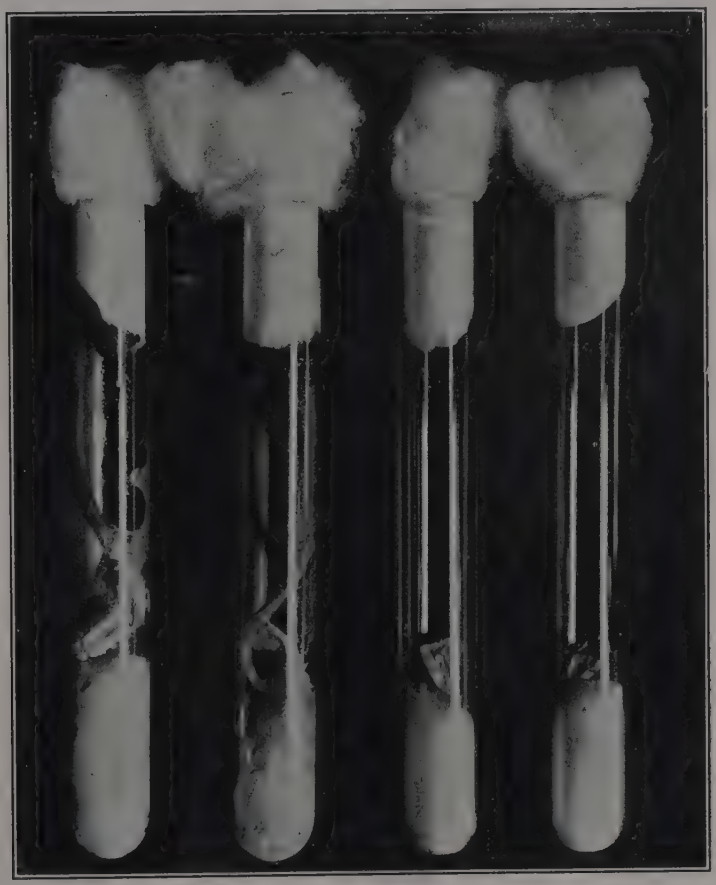


PLATE II.

Shows the method of germinating seed in sterilized test tubes containing absorbent cotton saturated with a sterile nutrient solution. Two-thirds natural size.

5. Pure cultures are made from the organisms developing from the infected seeds, and inoculation of the isolated organism into healthy plants both in tube cultures and in pot cultures in sand is also made.

Application of the Method.

1. Sampling the seeds—Representative samples were sorted out from the seeds collected. In each test an average of 25 to 35 seeds was used.

2. Sterilization of glassware—All petri dishes wrapped in paper, test tubes plugged with cotton and other glassware used in this investigation were sterilized in the hot air sterilizer for one hour at 150°C. All pots containing soils used in the experiments were first watered and then sterilized in a vertical autoclav (except when otherwise stated) for 3 hours at 120°C.

3. Preparation and sterilization of media[1]—The media used were nutrient agar and potato juice agar. The preparation and formula of each follows:

Nutrient agar—Prepared according to the following formula:

Agar	15 gms.
Sterile meat bouillon	900 cc.
Distilled water	300 cc.

The agar was cut into fine pieces and boiled in distilled water over a free flame until dissolved. The bouillon was then added to the agar and boiled for 20 minutes. It was allowed to cool to 45° to 50°C. and then the whites of three eggs were thoroughly mixed in. The mixture was heated over a water bath until the eggs were coagulated (30 min.). It was then filtered while hot through cotton or filter paper. The reaction was readjusted to +1.5, Fuller Scale.

Potato juice agar—Prepared according to the following formula:

Peeled potato	150 gms.
Agar	10 gms.
Distilled water	500 cc.

The potato was sliced into thin pieces and soaked in tepid water, allowing to simmer for half an hour. The juice obtained after filtering through cheese-cloth was used in place of bouillon. The remaining procedure was the same as the preparation of nutrient agar.

4. Preparation of cotton tubes—The cotton tubes were prepared by filling test tubes one inch with absorbent cotton which was saturated with a nutrient solution. Then the tubes were sterilized in an autoclav for one hour at 18 pounds pressure. The nutrient solution[2] used had an approximate osmotic pressure of 1.00 atmosphere and contained three salts in the following volume-molecular concentrations: Monobasic potassium phosphate (KH_2PO_4), .0022m; calcium nitrate ($Ca(NO_3)_2$), .0108m; and magnesium sulphate ($MgSO_4$), .0043m[3]. A trace of iron in the form of ferric phosphate ($FePO_4$) was added.

[1]The work was done in the laboratory of the Department of Bacteriology, and the writer wishes to acknowledge his indebtedness to Drs. E. M. Pickens and M. F. Welsh for their advice and co-operation during the preparation.

[2]The writer is indebted to Dr. Earl S. Johnston, Associate Plant Physiologist of this Experiment Station, for the preparation of this solution.

[3]The numbers indicate the fraction of the molecular weight of the salt in a liter of solution at about 20°C.

5. Germination of seeds and incubation of cultures—All the seeds and cultures were placed in a glass electric incubator for germination and incubation. Sometimes the germination of seeds, which were planted in the agar plates, was aided by placing them, in addition, in a moist chamber. The temperature of the incubator was varied between 20°-30°C., and recorded by a thermograph.

Presentation of Results.

ASPARAGUS (*Asparagus officinalis*).

Cylindrophora sp. (Plate III, figs. 1 & 2)—Seeds of apparently normal appearance were collected from decayed berries and treated (according to the previously described technique) for 3 minutes. The seeds were then planted on November 15, 1919, in cotton tubes and agar plates. Seeds germinated after seven days. Six seedlings grown in cotton tubes were found to have a white fungous growth upon them. The fungus was identified as *Cylindrophora sp.* It did not appear to be parasitic to the seedlings.

CABBAGE (*Brassica oleracea*).

No pathogens found—Sample 1 (large seed): Seeds of normal appearance were treated for periods of 2 and 3 minutes. All produced healthy seedlings three days after planting.

Alternaria sp. (Plate III, figs. 3 & 4)—Sample 2 (small seed): Seeds of normal appearance were subjected to the same treatment as sample 1. A species of *Alternaria* was found to inhibit the germination of seeds and to attack young seedlings which were grown in cotton tubes. The seedlings were wilted. During the experiment the writer was unable to note the presence of *Phoma lingam* which has been reported as being carried within the seed.

COMMON BEAN (*Phaseolus vulgaris.*)

Species of *Fusarium*, *Macrosporium*, and *Alternaria* were isolated from a sample of seeds which had marked symptoms of disease. The details are well shown in the following table:

Length of Time in Treatment.	*Color of Fungous Colony.*	*Fungi Identified.*	*Germination.*	*% of Seed Infected.*
2 minutes.....	Whitish Pink	Fusarium	None	90
3 minutes.....	Gray	Macrosporium	None	95
5 minutes.....	Dark Gray	Alternaria	None	95

The seeds were treated according to the previously described technique. The fungi had grown out from the hilum of the bean. An undetermined species of bacteria, which gave a striking coral red colored pigment, was observed in all the cultures in tubes.

PLATE III.

Fig. 1.—**Cylindrophora sp.** isolated from surface sterilized asparagus seed; fertile hyphae with spores. Magnified.

Fig. 2.—Conidia of **Cylindrophora sp.** Magnified.

Fig. 3.—**Alternaria sp.** found in the seeds of cabbage; fertile hyphae with spores. Magnified.

Fig. 4.—Conidia of **Alternaria sp.** Magnified.

COWPEA (*Vigna sinensis*).

Macrosporium sp. (Plate IV,figs. 1 and 2)—Samples of seeds with diseased appearance obtained from the Department of Agronomy were

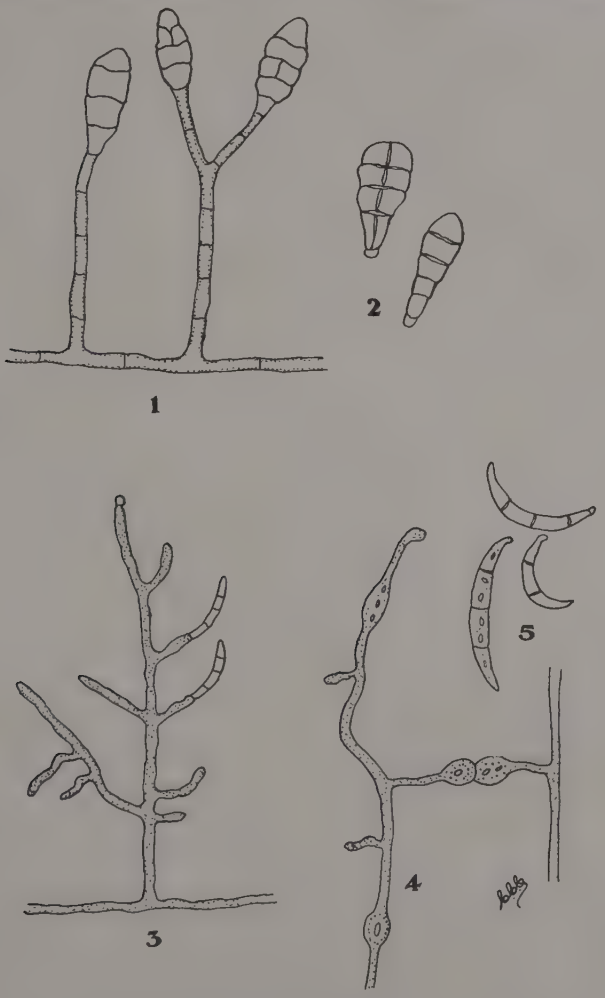


PLATE IV.

Fig. 1.—**Macrosporium sp.** isolated from surface sterilized cowpeas; fertile hyphae with spores. Magnified.

Fig. 2.—Conidia of **Macrosporium sp.** Magnified.

Fig. 3.—**Fusarium sp.** found in the seeds of Lima bean; fertile hyphae with spores.

Fig. 4.—Chlamydospores of **Fusarium sp.**

Fig. 5.—Conidia of **Fusarium sp.** Magnified.

treated for two different periods of 3 and 5 minutes. In one-half the cultures it was found that a dark gray fungus, *Macrosporium sp.*, had apparently prevented germination. Seedlings were obtained from the other half of the cultures, but all of them were also infected with the fungus. Apparently the fungus was not strictly parasitic to the host. However, the fungus gave the roots of seedlings a rusty appearance.

Lima Bean (*Phaseolus lunatus*).

Fusarium sp. (Plate IV, figs. 3, 4 & 5)—Seeds having much diseased symptoms were treated for 3 and 5 minutes and planted in cotton tubes and agar plates on November 12, 1919. A pinkish white mold growth had appeared on all the cultures one week later. None of the seeds germinated. The fungus was identified as a *Fusarium*. It had apparently grown out from the hilum of the seed.

Soybean (*Soja max*).

Macrosporium sp. and *Fusarium sp.*—These two fungi have been isolated from seeds having diseased symptoms. The pods were also covered with gray fungous spots. The experiment resulted thus:

Length of Time in Treatment.	*Color of Fungous Colony.*	*Fungi Found.*	*Germination.*	*% of Seed Infected.*
2 minutes.....	Black	Macrosporium	None	80
3 minutes.....	Black	Macrosporium	None	70
5 minutes.....	Pinkish	Fusarium	None	30

All plantings were made on April 26, 1920, and one week later it was noted that the fungi had grown out from inside the seed coat as tufts of mycelium.

Sweet Corn (*Zea saccharata*).

A fungous parasite of sweet corn seedling (Plate V, figs. 1 & 2)—While examining some harvested Stowell's Evergreen sweet corn ears in the Department of Vegetable and Floriculture early in January, 1920, it was noted that many kernels were infected with some kind of harmful organisms, giving a shriveled surface and discoloration of the grain.

A large number of diseased kernels were selected and taken to the laboratory. They were externally sterilized according to the previously described method for 3 minutes, and planted in agar plates and nutrient cotton tubes for germination under sterile conditions. One week later it was found that some kernels were infected with a white

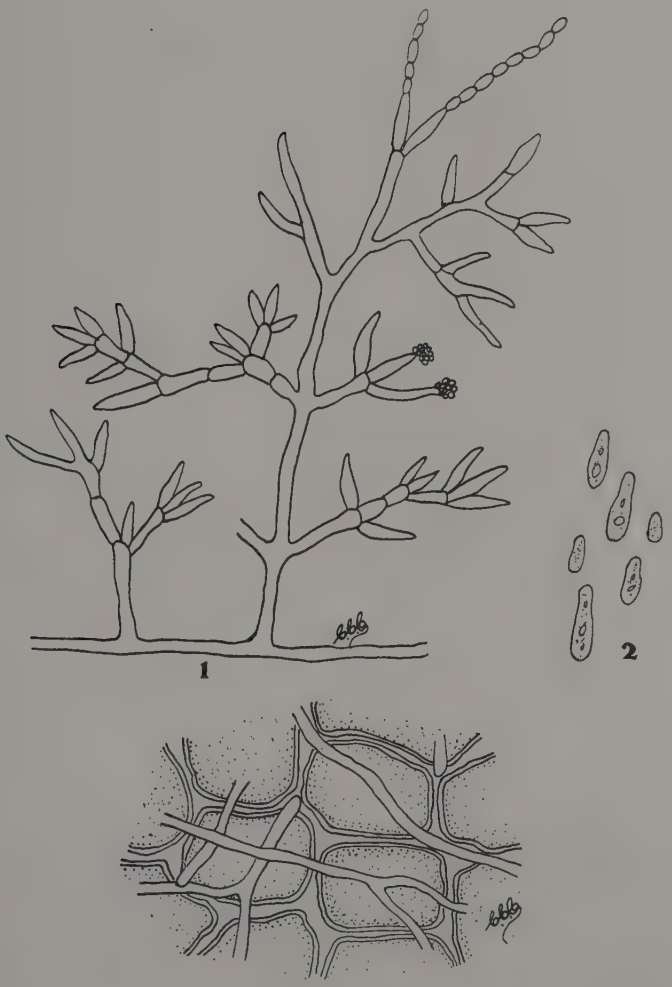


PLATE V.

Fig. 1.—A fungous parasite of sweet corn seedling; fertile hyphae with spores in chains and clusters. (Diagrammatic.)

Fig. 2.—Conidia of the fungus. Magnified.

Fig. 3.—Diagrammatic section showing the fungous mycelium in dead root tissue.

mold which had grown out from the inside of the kernels and apparently destroyed their contents, and some, although germinated, were also infected with the same fungus on their seedlings (Plate VI, fig. 1). The nutrient cotton tubes gave the best opportunity and facility to observe the fungous infection upon the growing seedlings (Plate VI, fig. 2). It was noted that the white fungus first begun to attack the tips of the roots, giving a brown discoloration, and gradually worked upward to the stem. The whole seedling was killed in about two weeks.

Pure cultures of the fungus were made on both nutrient agar and potato juice agar in tubes and plates. All of them served as good media for this fungous growth. On agar plates the erect fertile hyphae produced and the conidia formed on them can be clearly seen under a binocular. The formation of spores was observed within about 6 to 12 days. The fungus had the following characters:

Mycelium hyaline, elongated, branched; sterile hyphae creeping in the substratum; fertile hyphae erect, not branched, except for the short, often verticillate, side branches bearing the spores. Conidiophores straight, non-septate, hyaline, verticillate on lower part of fertile hyphae, alternate above, conidia in chains at the end of fertile hyphae and in clusters on the side of the fertile hyphae. Conidia oblong-ovoid, first in clusters and later in chains, hyaline, sometimes guttulate, 7.5-8.6-11.9 microns by 2.6-3.1-4 microns.

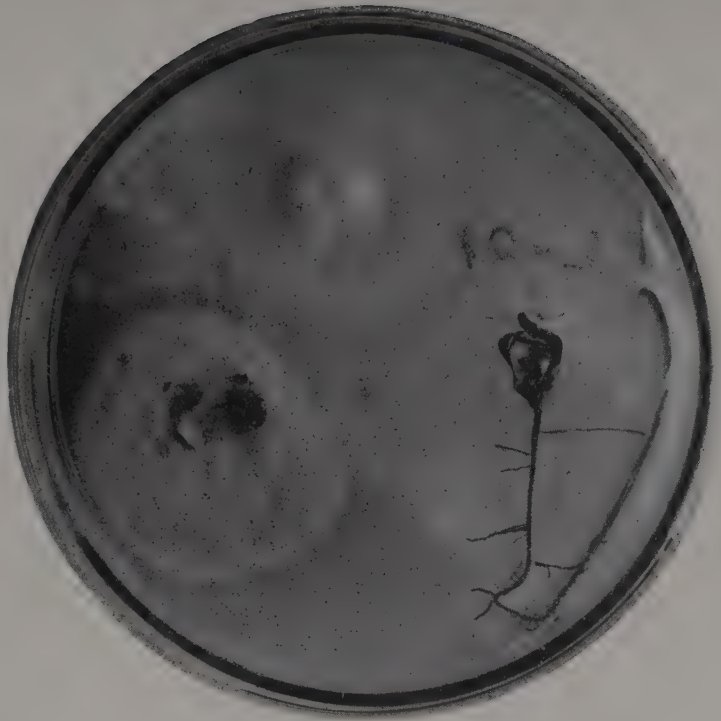
The fungus corresponds very well with the description of *Oospora verticillioides*, Saccardo, except that only the conidia in chains is mentioned by Saccardo (80). His description of the fungus follows:

"Caespitulis minutis albidis; hyphis sterilibus repentibus, ramulos fertiles simplices v. furcatos v. subverticillatos 70x3 continuos exserentibus; conidiis breiter catenulatis oblong-fusoidois v. subobclavatis, 8-10x2.5-3, hyalinis, quandoque guttulatis.

"Hab. in caryopsidibus maceratis Zeae Maydis, Padova et Conegliano Italiae borealis."

Oospora verticillioides was extensively studied by Tiraboschi (86) in an investigation of organisms in corn that might be connected with pellagra. Tiraboschi, like practically all other students of corn diseases, apparently overlooked similar work done in Russia in 1895 and 1896 by Deckenbach (25, 26), who in addition found that *Oospora verticillioides* was parasitic on corn. After Tiraboschi's paper was published, Deckenbach (27) again reviewed his original work in 1907.

It is probable that this fungus has been recorded under other generic names by some writers. *Cephalosporium sacchari*, described by Butler (21) as a sugar cane parasite in India, accords very well with this fungus, except that the conidia in chains were not noted by him. The distinctions between *Cephalosporium*, *Acrostalagmus*, *Verticillium* and similar genera are slight, and as the chains of spores of this fungus are not always easily found, this corn parasite may sometimes have been classed in one of these genera. During the study of this fungus the writer (71) was unable to note the presence of a *Fusarium* stage, but



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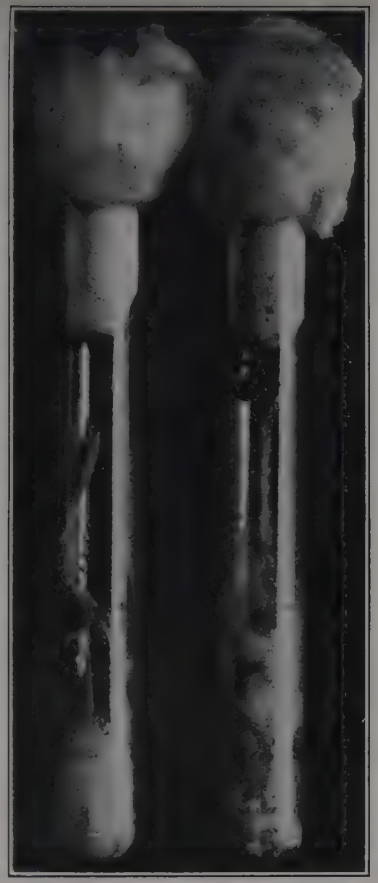

2.

PLATE VI.

Fig. 1.—Shows the internal fungous infection upon surface sterilized sweet corn grains grown in sterile nutrient agar plate.

Fig. 2.—Shows infection by the fungous parasite upon growing sweet corn seedlings in tubes.

if it should develop the parasite would have to be referred to *Fusarium moniliforme,* described by Sheldon (82) in 1903. This fungus was further reported by Valleau (87) in 1920 as the cause of the root infection of corn plants in the field.

To determine the pathogenicity of this fungus a series of inoculations was conducted. Healthy seedlings of both sweet corn and field corn grown in nutrient cotton tubes were inoculated with the fungus from the pure culture. They were all infected and killed in a time varying from 5 to 9 days. Thirty healthy plants of sweet corn were grown in the greenhouse in 6-inch pots (soils not sterilized; 3 plants in each pot) under conditions as nearly normal as possible. When the seedlings were 7-8 cm. high, inoculations were made as follows: 15 plants as control; 6 plants inoculated by pouring in around the root a spore suspension from a tube culture, and 9 plants inoculated by puncturing the stem just above the root with a sterile needle infected with a bit of fungous mycelium and spores from a pure culture. A similar inoculation test was made with field corn plants. The planting was started on April 19, inoculations made on May 3, and the experiment concluded on May 21. About 7 days after inoculation the plants with puncture inoculations had developed four cases in field corn and three cases in sweet corn, of distinct dwarfness and distortions. Such symptoms had been noticed in the field by Mr. T. H. White, in charge of Vegetable and Floriculture at the Station, who grew some sweet corn of the same variety last season. He has every reason to believe that this fungus was responsible for the barren stalks, root rot, and dwarfness occuring in the corn grown last season, but the writer was unfortunately not able to make a more extensive test in the field, owing to the limitation of time. The effect of inoculation on the height of the corn plants is shown in the following table:

AVERAGE IN HEIGHT OF CORN IN CM.

Kind.	*Control.*	*Inoculated with Spore Solution.*	*Inoculated by Puncturing.*
Sweet corn	52	47	42.8
Field corn	45.7	38	36.1

These striking results are shown further in Plate VII, figs. 1, 2 and 3.

In examining some of the distorted or stunted plants by cutting lengthwise through the base, the tissue seemed to have a marked brown discoloration. A section mounted under the microscope showed the presence of fungous mycelium in the cells of the decayed tissue (Plate V, fig. 3). The brown parts of the root of an infected plant were also externally sterilized and placed in agar plates for incubation. The same fungus was recovered from them.

In connection with this fungous parasite of sweet corn, it might be interesting to note the recent reports on the same subject by Hoffer and Holbert (55, 56, 58). They state that harmful organisms, such as species of *Gibberella, Fusarium, Verticillium, Rhizopus,* and *Pseudomonas,* although the technical description of them are not given, are responsible for missing hills, slow growing stalks, barren stalks, and rotting of the embryo. They (57) also thought that the common wheat scab organism, *Gibberella saubinetii,* is probably the most common pathogen responsible for the root and stalk rotting of corn plants. In addition, they recommend the ear-to-row method for studying the quality and value of seed ears, selection of seed ears from disease-free stalks, and the use of the germinating box for discarding seedlings from stock carrying disease-producing organisms as possible means of con-

PLATE VII.

Fig. 1.—Corn seedlings inoculated by puncturing the stem just above the root with a sterile needle infected with a bit of the mycelium of sweet corn seedling parasite. A case of distortion shown in one plant. Photographed 18 days after inoculation.

Fig. 2.—Control plants. Photographed at the same time as the inoculated plants.

Fig. 3.—Corn seedlings inoculated by pouring in the spore solution from the pure culture of the fungous parasite around the root system. Photographed 18 days after inoculation.

trol. Duddleston (28) of the Purdue Station more recently recommends the modified rag doll seed tester for controlling corn infecting organisms by a study of the germinating seedlings. These recommendations are worthy of consideration in developing methods of controlling the present fungous parasite.

Tomato (*Lycopersicum esculentum.*).

Mosaic—Seeds collected from mosaic diseased plants were planted in pots of sterilized soil on November 26, 1919, and 40 plants were secured from the seeds. Seeds from plants without mosaic were also planted as checks. The plants were placed in a greenhouse which was free from mosaic plants and insects. These plants were kept under observation until February 2, 1920, but they showed no mosaic symptoms. They were then inoculated by puncturing the stem with a needle infected with mosaic leaf tissue, and also by rubbing their leaves with crushed mosaic leaf. At that time the plants had developed their sixth leaves. Three weeks later they all showed the characteristic symptoms of mosaic, narrow, distorted and even filiform young leaves.

Rhizopus nigricans (Plate VIII, figs. 1 and 2)—That the seed from rotten tomatoes often has a dark color and may be injured by rot organisms had been reported by Norton and Leathers (69, p. 202) in 1918. They also stated that such seeds gave a poor germination. In the present experiment seeds from similar rotten tomatoes were used and divided into two parts for treatment (3 and 5 min.) according to the previously described technique. The treated seeds were planted in both cotton tubes and agar plates on November 14, 1919. Three weeks later the seedlings of 3-minutes treatment were found dead, and a gray colored mold had grown over them. The fungus was identified as *Rhizopus nigricans*, Ehr. To determine the pathogenicity of this fungus, ten inoculations were made on healthy seedlings grown in cotton tubes. In all cases the inoculated seedlings were killed 7 days later. Four inoculations were made on young plants (4th leaf developed) grown in pots in the greenhouse, but a negative result was obtained. No fungous infection was found on the seedlings of 5 minutes treatment.

Wheat (*Triticum aestivum*).

A sterile fungus (Plate VIII, fig. 3)—Shriveled wheat grains were treated for 3 minutes and planted in cotton tubes and agar plates. The seedlings grown in tubes were infected by a white fungus, which soon caused their death. A series of inoculations was made on healthy seedlings grown in tubes, and positive results were obtained. It was noted that the fungus first attacked the root system of the host and then gradually reached the stem. Pure cultures were made on nutrient agar, potato juice agar, and tomato stem. A vigorous growth of the fungus was noted on all of the media. The fungus was kept under observation over 5 months, but no conidia developed. The fungus in

colony was a felt-like white mass. Its mycelium was hyaline, non-septate, and much branched. Many large chlamydospores developed in the mycelium.

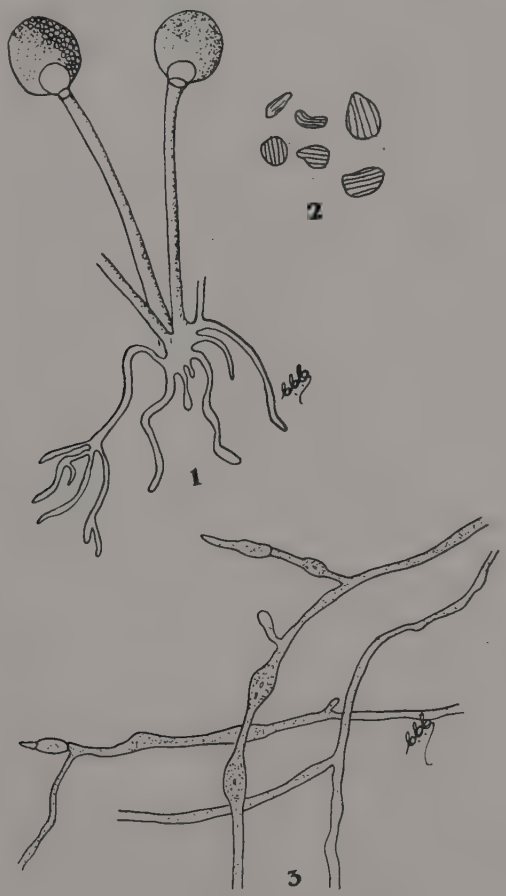

PLATE VIII.

Fig. 1.—**Rhizopus nigricans,** Ehr. isolated from dark seeds of rotten tomato; fertile hyphae with spores.

Fig. 2.—Conidia of **Rhizopus nigricans.** Magnified.

Fig. 3.—A sterile fungus isolated from wheat seedlings; mycelium with thick wall chlamydospores. Magnified.

Seeds Tested But No Pathogens Found.

Seeds of the following crops were tested, but no pathogens were found: Barley, alfalfa, timothy, red clover and crimson clover.

CONCLUSION AND DISCUSSION.

The literature cited in this paper and the experiments described show that some of the fungi and bacteria occurring in the seeds of host plants are pathogenic when, under favorable conditions, they come in contact with seedlings, while only a few forms apparently are non-parasitic. Their identity, as well as their pathogenicity to the host and the extent to which they affect crops should be ascertained by laboratory, greenhouse and field tests. These need to be reinforced by pure culture inoculations of seeds, seedlings and plants in various stages of growth under natural conditions before the exact relation of such internal parasites of seeds to the host can be definitely established.

This present paper has well demonstrated that the internal parasites of agricultural seeds have a direct bearing upon the production of crops. The subject is especially important when food production is imperative. Our most valuable foods consist of seeds, and healthy plants are developed from the sound seeds. With these points in view, there is an urgent need for a study of the parasites in seeds generally, as well as of their relation to crops. The present work is a preliminary survey of this question, but it is hoped that this preliminary work may be of value to farmers and investigators who are interested in the problems of healthy seed. It seems to the writer that if a more detailed and far-reaching study were made of this subject, it would be very promising in valuable additions to our knowledge of seed and seedling troubles.

The control of internal parasites of seeds is rather difficult. Ordinary seed treatment will not destroy these internal pathogens without destroying the vitality of the seed. However, seed selection from disease-free plants and planting seeds free from disease symptoms can be considered as practical means of control. Hot water and hot air are the most effective treatments for internal pathogens, providing the thermal death point of the pathogens is lower than that of the seed. A more critical study of the results of the germination test can be made to detect the primary infection upon seeds and seedlings by pathogens. Through further studies it will be possible to develop practical means to control internal parasites of agricultural seeds.

LITERATURE CITED.

1. Anonymous. Plant diseases in Ireland. Dept. Agr. and Tech. Instr. Ireland, Ann. Gen. Rpt. 18:60. 1917-18.
2. Barre, H. W. Cotton anthracnose. S. Carolina Sta. Bul. 164: 11-15. 1912.

3. ————————. Cotton anthracnose. S. Caroline Sta. Rpt. 22:89. 1912.
4. Barrus, M. F. The dissemination of disease by means of the seed of the host plant. Proc. Ind. Acad. Sci. 1908:113-122.
5. Beach, S. A. Some bean diseases. N. Y. State Sta. Bul. 48:318. 1892.
6. Beauverie, J. The occurrence of rust spores in the interior of seeds of grasses. Compt. Rend. Acad. Sci. (Paris) 157:787-790. 1913.
7. ————————. Rust in the interior of graminaceous seeds. Rev. Gen. Bot. 25:11-27. 1914.
8. ————————. The efficiency of rust spores contained in seed of grain for the propagation of disease. Compt. Rend. Acad. Sci. (Paris) 158:1196-1198. 1914.
9. Blaringhem, L. A preliminary note on the heredity of fungus diseases of certain plants. Bul. Soc. Bot. France 59:217-220. 1912.
10. ————————. Transmission of diseases by seeds. Assoc. Franc. Avanc. Sci: Compt. Rend. 43:470-478. 1914.
11. Bolley, H. L. Seed disinfection, etc. N. Dakota Sta. Bul. 87: 142-144. 1910.
12. ————————. Wheat. N. Dakota Sta. Bul. 107:81-83. 1913.
13. Bolley, H. L. and F. J. Pritchard. Internal infection of the wheat grain by rust. Science, n. s., 22:343. 1905.
14. ————————————. Rust problems. N. Dakota Sta. Bul. 68. 1906.
15. Bondarstev, A. S. A new disease of the flowers of red clover and its relation to seed production. Zap. Sta. Isp. Siem. Imp. Bot. Sad. 2. 1914.
16. Braun, H. Presoaking as a means of preventing seed injury due to disinfectants and of increasing germicidal efficiency. Science, n. s., 49:544-545. 1919.
17. Braun, H. Presoak method of seed treatment. Jour. Agr. Research, 19:363-392. 1920.
18. Brefeld, O. New investigations and results on natural infection and distribution of grain smuts. Nachrichten aus dem Klub der Landwirte zu Berlin, No. 466:4224-4234. 1903.
19. ———————— and R. Falck. Flower infection by smuts. Untersuch. Gesam. Geb. Mykol. No. 13. 1905.—also abs. in Bot. Centbl., 101:212. 1906.
20. Burrill, T. J. and J. T. Barrett. Ear rots of corn. Ill. Sta. Bul. 133. 1909.
21. Butler, E. J. Mem. Dept. Agri. India Botan. Series. 6:181-190. 1913.
22. Cayley, Dorothy M. A preliminary note on a new bacterial disease of Pisum sativum. Proc. Royal Soc. (London) Ser. B. 86: 171-173. 1913.
23. Clinton, G. P. Report of the Botanist. Conn. Agr. Expt. Sta. Rpt. 1905:289-293.

24. Cook, M. T. Common diseases of garden vegetables and truck crops. N. J. Sta. Circ. 89:19. 1918.
25. Deckenbach. Compt. rendus Soc. Imp. Nat. St. Petersburg, 1896: 130-131.
26. ————————. Scripta botanica Horti. Universitatis Petropol, 15:45. 1898. Also 15:101. 1899.
27. ————————. Centr. Bakt., 1 Abt. Originale, 45:507-512. 1907.
28. Duddleston, B. H. The modified rag doll and germinator box. Purdue Sta. Bul. 236. 1920.
29. Eriksson, J. The latent life of the Uredineae. Compt. Rend. Acad. Sci. (Paris), 124:475-477. 1897.
30. ————————. General review of the principal results of Swedish research on grain rusts. Bot. Gaz. 25:26-38. 1898.
31. ————————. The origin and propagation of cereal rusts through the seed. Ann. Sci. Nat. Bot., 8 ser., 15:1-160. 1902.
32. ————————. The hollyhock rust, its nature and developmental phases. Compt. Rend. Acad. Sci. (Paris) 152:1776-1779. 1911.
33. ————————. Investigations on hollyhock rust. Centbl. Bakt. (etc.), 2. Abt., 31:93-95. 1911.
34. ————————. On the appearance of spores and mycelium of rust within the grains of cereals. Compt. Rend. Acad. Sci. (Paris), 158:1194-1196. 1914.
35. ————————. Beet rust. Rev. Gen. Bot. 25 bis:247-258. 1914.
36. ————————. New diseases of cucumber in Sweden. Centbl. Bakt. (etc.), 2. Abt., 44:116-128. 1915.
37. Faulwetter, R. C. The angular leaf spot of cotton. S. Carolina Sta. Bul. 198. 1919.
38. Frank, A. B. Berichte der deutsch. Bot. Ges. 1:31. 1883.
39. ————————. Die Krankheiten der Pflanzen. 2:380:382. 1896.
40. Freeman, E. M. The seed fungus of the darnel. Proc. Roy. Soc. (London), 71:27-30. 1902.
41. ————————. Symbiosis in Lolium (abstract). Science, n. s., 19:172. 1904.
42. ————————. Symbiosis in the genus Lolium. Minn. Bot. Studies, 3 ser. pt., 3:329-334. 1904.
43. ———————— and E. C. Johnson. The loose smuts of barley and wheat. U. S. D. A. Bur. Plant Indus. Bul. 152. 1909.
44. Galippe, V. Parasitism of seeds. Compt. Rend. Acad. Sci., 161: 112-115. 1915.
45. Gilbert, W. W. Cotton anthracnose and how to control it. Farmers' Bul. 555:4-6. 1913.
46. Guerin, P. The probable cause of the poisonous effects of the darnel. Bot. Gaz., 28:136-137. 1899.
47. Halsted, B. D. The anthracnose of the bean—remedy suggested. N. J. Sta. Annual Rpt., 1891:284-287.
48. ————————. A bacterium of Phaseolus. N. J. Sta. Ann. Rpt., 1892:283-286.

49. ————————. Anthracnose in bean seeds. Garden and Forest, 5:18. 1892.
50. ————————. Some fungous diseases of the pea. N. J. Sta. Rpt., 1893:357-362.
51. Harter, L. L. Pod blight of the Lima bean caused by *Diaporthe phaseolorum*. Journ. Agr. Research, 11:473. 1917.
52. Heald, F. D. The dissemination of fungi causing disease. Amer. Micros. Soc. Trans., 32:5-29. 1913.
53. Hecke, L. An internal infection germ of cereal smuts. Ztschr. Landw. Versuchsw. Oesterr., 7:59-64. 1904.
54. Henderson, M. P. The black-leg disease of cabbage caused by *Phoma lingam* (Tode) Desmaz. Phytopath., 8:379-432. 1918.
55. Hoffer, G. N. Disease-free sweet corn seed. Purdue Sta. Bul. 233. 1920.
56. ———————— and J. R. Holbert. Selection of disease-free seed corn. Purdue Sta. Bul. 224. 1918.
57. Holbert, J. R. and G. N. Hoffer. Corn root and stalk rots (abstract). Phytopath., 10:55. 1920.
58. ————————————. Control of the root, stalk, and ear rot diseases of corn. U. S. D. A. Farmers' Bul. 1176. 1920.
59. Hopkins, E. F. Overwintering and control of bur clover leaf spot (abstract). Phytopath., 10:66. 1920.
60. Hori, S. Seed infection by smut fungi of cereals. Bul. Imp. Cent. Agr. Expt. Sta. Japan, 1:163. 1907.
61. Hungerford, C. W. Rust in seed wheat and its relation to seedling infection. Jour. Agr. Research, 19:257-278. 1920.
62. Jaczewskii, A. A. Fungus and bacterial diseases of clover. Min. Zeml. Biuro. Mikol. Fitopath. Uchen. Kom. 1916.
63. Krueger, F. An unusual appearance of *Ascochyta pisi* upon peas. Centbl. Bakt. und Par. Allg., 1:620-624. 1895.
64. Maddox, F. Experiments at Eastfield. Dept. of Agr., Tasmania. 1895.
65. ————————. Notes and results on agricultural experiments carried on under the auspices of the council of agriculture of Tasmania at Eastfield, Newnham. Launceston, Tasmania. 1897.
66. Massee, G. The cereal rust problem—does Eriksson's mycoplasma exist in nature? Nat. Sci., 15:337-346. 1899.
67. Miura, S. On the grain of barley or wheat, infected by smut fungus through the flower. Ann. Phytopath. Soc. Japan, 1:16-26. 1918.
68. Nishikado, Y., and C. Miyake. Disinfection of rice grain for the control of the brown spot disease. Byo-chu-gai Zasshi (Journ. Plant Protec.), 59:1-20. 1918.
69. Norton, J. B. S. and C. E. Leathers. Conditions detrimental to seed production. Md. Sta. Bul. 216. 1918.
70. ———————— and C. C. Chen. Some methods for investigating internal seed infection. Phytopath., 10:399-400. 1920.

71. ———————— and C. C. Chen. Another corn seed parasite. Science, n. s., 52:250-251. 1920.
72. O'Gara, P. J. A podosporiella disease of germinating wheat. Phytopath., 5:323-326. 1915.
73. Osner, G. A. Leaf smut of timothy. Cornell Sta. Bul. 381. 1916.
74. Pammel, L. H., et al. Studies on a Fusarium disease of corn and sorghum. Iowa Sta. Research Bul. 33. 1916.
75. Pethybridge, G. H., and H. A. Lafferty. A disease of flax seedlings caused by a species of Colletotrichum and transmitted by infected seed. Sci. Proc. Roy. Dublin. Soc., 15:359-384. 1918.
76. Pritchard, F. J. A preliminary report on the yearly origin and dissemination of *Puccinia graminis* in N. Dakota. Botanical Gazette, 42:169-192. 1911.
77. ————————. The wintering of *Puccinia graminis tritici,* E. & H. and the infection of wheat through the seed. Phytopath., 1:150-154. 1911.
78. Reddick, D. and V. B. Stewart. Transmission of the virus of bean mosaic in seed and observations on thermal death-point of seed and virus. Phytopath., 9:443-451. 1919.
79. Rolfs, F. M. Angular leaf spot of cotton. S. Carolina Sta. Bul., 184:14-17. 1915.
80. Saccardo, P. A. Sylloge Fungorum., 4:14. 1886.
81. Selby, A. D and T. F. Manns. Studies in diseases of cereals and grasses. Ohio Sta. Bul. 203. 1909.
82. Sheldon, J. L. A corn mold. Nebr. Sta. 17th Rpt., 1903:23-32.
83. Smith, W. G. Corn mildew. Gard. Chron., n. s., 24:245. 1885.
84. ————————. Corn mildew and barberry blight. Gard. Chron., n. s., 25:309-310. 1886.
85. Stewart, F. C. A bacterial disease of sweet corn. New York Sta. Bul. 130:437. 1897.
86. Tiraboschi, C. Sopra alcuni Ifomiceti del mais quasto di regioni pellagrose. Annali di Botanica, 2:137-168. 1905.
87. Valleau, W. D. Seed corn infection with *Fusarium moniliforme* and its relation to the root and stalk rots. Ky. Sta. Bul. 226. 1920.
88. Van Hook, J. M. Blighting of field and garden peas, and powdery mildew of the pea. Ohio Sta. Bul. 173. 1906.
89. Westerdijk, J. Die mosaikkrankheit der tomaten. Mededeelingen uit het Phytopathologish Lab. Willie Commelin Scholten, Amsterdam, 1:5-20. 1910.
90. Whetzel, H. H. Some diseases of beans. Cornell Sta. Bul. 239. 1906.
91. Wolf, F. A. Dissemination of bur clover leaf spot. Phytopath., 6:301. 1916.
92. Zukal, Hugo. Research on the grain rusts in Austria-Hungary (Series 1). Sitzber. K. Akad. Wiss. (Vienna), Math. Naturw. Kl., Abt. 1, Bd. 108. Heft. 6-7:543-562. 1899.

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